The impact of long-term water stress on tree architecture and production is related to changes in transitions between vegetative and reproductive growth in the 'Granny Smith' apple cultivar

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Running title: Impact of water stress on apple tree architecture

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Abstract

Water stress generates a number of physiological and morphological responses in plants that depend on the intensity and duration of stress as well as the plant species and development stage. In perennial plants, water stress may affect plant development through cumulative effects that modify plant functions, architecture and production over time. Plant architecture depends on the fate of the terminal and axillary buds that can give rise, in the particular case of apple, to reproductive or vegetative growth units of different lengths. In this study, the impact of long-term water stress (7 years) on the fate of terminal and axillary buds was investigated in relation to flowering occurrence and production pattern (biennial vs. regular) in the 'Granny Smith' cultivar. It was observed that water stress decreased the total number of growth units (GU) per branch, regardless of their type. Conversely, water stress did not modify the timing of the two successive developmental phases characterized by the production of long and medium GUs and an alternation of floral GUs over time, respectively. The analysis of GU successions over time using a variable-order Markov chain that included both the effects of the predecessor and water treatment revealed that water stress reduced the transition towards long and medium GUs and increased the transition toward floral, short and dead GUs. Water stress also slightly increased the proportion of axillary floral GUs. The higher relative frequency of floral GUs compared to vegetative ones reduced the tendency to biennial bearing under water stress. The accelerated ontogenetic trend observed under water stress suggests lower vegetative growth that could, in turn, be beneficial to floral induction and fruit set.

Introduction

Plants subjected to water stress respond by a number of physiological mechanisms at the molecular, cellular, organ and whole plant levels. These responses depend on the intensity and duration of water stress, as well as on the plant species and plant development stages (Müller and Leyser 2011). Under water stress, both water relationships and carbon balance are impaired via stomata closure that limits water loss by transpiration and, consequentially, photosynthesis activity (Hsiao 1973). Alterations of the internal metabolism of plants under water stress are accompanied by modifications in shoot structure and organ size, thus providing the plant with the flexibility to respond to water stress to produce a suitable architecture for the climatic context in which it grows (Hsiao 1973, Ferguson and Beveridge 2009). Evidence can be found from numerous studies that water stress results in the reduction of the growth rate of leaves and shoots, leaf area, shoot length and branching, leading to a shortened plant life cycle and an early transition from the vegetative to the reproductive stages. These responses have been observed for crop, herbaceous, and forest plants (Aronson et al. 1992, Borchert 1994, Lebon et al. 2006, Tardieu et al. 2011).

Fruit trees are usually irrigated and their growth and production heavily depend on water availability and irrigation in many countries (Mills et al. 1996, Mpelasoka et al. 2001). Nevertheless, water stress has become a critical problem in fruit tree orchards, especially in arid and semi-arid regions (Naor et al. 2008, Fernandez and Cuevas 2010). In such situations, a lower irrigation supply decreases mean apple fruit mass, irrespective of fruit load (Naor et

al. 2008). Water stress has also been shown to cause reductions in the number of organs produced each year (metamer number per shoot) in mature peach (Girona et al. 2005) and olive (Martín-Vertedor et al. 2011). However, this may depend on the tree age and shoot type since it has been reported that the metamer number is unaffected by water stress in first-order shoots of one-year-old peach trees (Hipps et al. 1995) and in epicormic shoots in almond (Negrón et al. 2014). The analysis of branching characteristics in almond also showed that specific zones of branching along shoots were reduced in length or even absent after water stress, but the effect was minor on axillary flowers. In grapevine, first-order shoots were practically not affected by moderate water stress, whereas the number of metamers on sylleptic branches was reduced. Under severe stress, both types of shoots were affected (Lebon et al. 2006, Pallas and Christophe 2015).

In apple tree, flower density can be drastically reduced under early summer water stress because of the lag period between flower induction that occurs approximately 60 days after full bloom (Buban and Faust 1982, Foster et al. 2003) and flowering the following spring. Such a reaction was found in the absence of irrigation during postharvest in almond (Goldhamer and Viveros 2000). This suggests that the effect of water stress over time should be taken into account in perennial plants, as should the modifications it generates in plant architecture due to the regulation of shoot growth, branching and flowering from one year to the next. Most of the studies that have been conducted so far on tree responses to water stress have focused on modifications in shoots, leaves and fruits after treatments applied within a single growing season. Less attention has been paid to the cumulative effect of water stress on the tree architecture and its development during consecutive years, probably

because of the difficulty to analyze tree architecture and to carry out long-term experiments that are needed to observe cumulative, acclimation and compound effects on tree structure and functions (Myers 1988, Chaves et al. 2002, Bréda et al. 2006).

Plant architectural analysis provides a framework to perform studies in which plant development can be considered as the result of the production of repeated elementary structures (White 1979), even though the number and types of elementary structures produced during tree ontogeny have been shown not to be exactly similar over time due to morphogenetic gradients (Barthélémy and Caraglio 2007). One of the most obvious morphogenetic gradients is the decrease in the length of growth units (GUs) with tree age, which has been observed in many trees and, in particular, in apple tree. These gradients also concern branching characteristics that accompany GU length changes with tree age (Renton et al. 2006). Based on early studies that revealed the existence of heteroblasty in most fruit trees (see Costes et al. (2006) for a review), four types of GUs were taken into account to describe and analyze these gradients, considering both the GU length and their number of metamers (Costes et al. 2003, Costes et al. 2008, Costes and Guedon 2012). Vegetative GUs longer than 20 cm include preformed and neoformed metamers with elongated internodes. Medium GUs are between 5 and 20 cm long and consist mainly of preformed metamers with elongated internodes, but may also include some neoformed metamers. Short GUs are shorter than 5 cm and are constituted of non-elongated preformed metamers. Floral GUs (also called ‘bourses’) result from floral differentiation of the apical meristem and consist of both vegetative preformed metamers (approximately 7) (Costes 2003) with non-elongated internodes in the proximal part and flowers in the distal part (Crabbé 1987). Floral GUs

usually have five flowers and can bear from zero to five fruits depending on fruit set. Floral GUs usually bear one or two sympodial neoformed vegetative GUs (referred to as 'bourse shoots'), which develop immediately (Crabbé and Escobedo-Alvarez 1991).

Based on this description of GU types, the changes that occur during tree ontogeny in the GU succession along the axes have been modeled as successive stages by variable-order Markov chains (VOMCs), including the occurrence of floral GUs (Costes and Guédon 2012). Moreover, several indexes, inspired from the Biennial Bearing Index (Wilcox 1944) and previous studies dedicated to the study of the fruiting behavior of fruit trees (Lauri et al. 1995, Cilas et al. 2011), have been proposed to characterize both the regularity (vs. bienniality or irregularity) and the synchronicity of flowering occurrences in young trees that exhibit increasing fruit production (Durand et al. 2013). These studies have shown that the development of GUs changes depending on their position within the tree structure and on the stage of plant development. These changes then affect the fruit yield. However, in these studies, branch and axis structures and tree architecture were described under optimal watering conditions.

Our study aimed at analyzing the effect of water stress over time at different scales of plant organization (whole tree, branch, axis and GU) in apple tree. The experiment was carried out on trees of the 'Granny Smith' cultivar grown under long-term soil water stress, i.e., applying summer soil water stress for 7 years. Our hypothesis was that such a long-term water stress would modify the composition of shoot types (vegetative vs. reproductive, long vs. short GUs) within a branch, with potential repercussions at the whole-tree scale on the fruit production pattern (regularity vs. irregularity). The following questions were addressed:

(1) Can a decrease in primary growth (GU length) be observed in response to water stress? (2) Are the inter-annual transitions between GUs modified by water stress? (3) Does water stress modify the floral GU frequency and production patterns at the tree scale?

Materials and methods

Plant material

In February 2007, sixteen 'Granny Smith' trees were grafted onto M9 rootstock and planted at the DiaScope INRA Experimental Station near Montpellier (France, N43°36, E03°58). 'Granny Smith' has a weeping growth habit with long shoots, exhibits fruit-bearing regularity, and belongs to the type IV tree architecture (Lespinasse 1992). 'Granny Smith' trees were included in a plot composed of approximately 520 trees corresponding to a progeny issued from a 'Starkrimson' × 'Granny Smith' cross (Virlet et al. 2014). In the plot, trees were planted along ten rows, with an inter-row distance of 5.0 m, a within-row distance of 2.0 m, and a northwest-southeast orientation. A 2.5 m wide alley crop of tall fescue (*Festuca arundinacea*) was sown in April 2007 between rows. Phytosanitary treatments and fertilizing were performed by conventional means, consistent with professional practices. Fruit thinning was carried out each year according to professional practices, i.e. limiting to one the number of fruits per inflorescence. Fruit thinning intensity was identical on each tree of the experiment. The trees were not pruned during the experiment. Throughout the experiment (2007-2014), mean daily air temperature, mean daily maximal VPD and mean daily solar radiation during the growing periods (1 April–15 September) were similar among

years and were equal to 20.4 °C (+/- 0.44), 2.00 kPa (+/- 0.22) and 22.3 MJ m^{-2} (+/- 0.63), respectively (Supplementary Data, Figure S1). Conversely, total rainfall during the growing period was more variable among years with values ranging from 123 mm (2011) to 237 mm (2009).

Water stress treatment

The experimental plot was organized in five rows with well-watered trees (WW) alternated with five other rows of trees submitted to water stress (WS). Eight trees per treatment were studied. WW trees were irrigated using a system of microsprayers located in the rows, with one 20 l h^{-1} emitter per tree. Watering management was performed as previously described by Virlet et al. (2014). Irrigation of the WW trees was scheduled to avoid any soil water depletion and WS treatment was applied from 2007 to 2013 (no water stress in 2014). WS trees had the same water supply as WW trees before summer. Watering was then suppressed from the end of June to the end of August, resulting in a progressive increase in water stress during summer. Soil water potential was recorded with 12 Watermark sensors connected to a Monitor datalogger (Irrometer Company, Inc., Riverside, CA, USA), located in a representative area of the field on WW and WS trees, and placed at a depth of 30 cm. This setup allowed the automatic acquisition of soil water potential four times a day throughout the experiment. These automatic data measurements were supplemented by some manual measurements with the same Watermark sensors from 2011 to 2013.

Phenotyping

Whole tree variables - Six trees per treatment were described during the experiment because two trees per group either died or were damaged. From 2010 (first year of production) until 2014, the yield and the number of fruits were recorded for each tree. Mean individual fruit weight was estimated as the ratio between yield and the number of fruits per tree. From 2011 until 2013, the basal trunk diameter was also recorded because it is considered as a good indicator of tree vegetative vigor (Strong and Azarenko 2000). The cross-sectional area was estimated assuming a cylindrical shape for the trunk.

Branch architecture description - In May 2014, all the branches arising from the first and second annual GUs of the trunk and that had not been broken during the experiment were selected and analyzed for each tree. From one to five branches per tree were studied, depending on the initial variability in tree architecture and the number of broken branches.

The morphological description of these branches was performed using morphological markers such as leaf scars (Barthélémy and Caraglio 2007), which made it possible to analyze *a posteriori* their structure over time. The branch topology was coded in the form of multiscale tree graphs (MTG, Godin and Caraglio 1998), considering four scales: branch, axis, growth unit (GU) and metamer (Costes et al. 2003). Four types of GUs were defined: Long GUs (designated as L, length > 20 cm); Medium GUs (designated as M, 5 cm < length < 20 cm); Short GUs (designated as S, length < 5 cm) and Floral GUs (designated as F). When more than one bourse shoot arose from the same floral GU, the longest one was chosen as the axis continuation. Bourse shoots were also designated according to their length (Long, Medium, Short). A GU was considered dead (designated as D) if it did not produce any new GUs in the terminal position before the end of the experiment.

GU type successions were recorded on the second- and third-order axes (see Supplementary Data, Figure S2). The number of metamers per GU was recorded for long and medium GUs belonging to second-order axes. The fate of all the axillary meristems was also recorded for those GUs, considering the four GU types previously defined (long, medium, small and floral) or the latent bud if no ramification was observed.

Data analysis

Defining variables of interest for analyzing branch architecture and growth unit succession - The VPlants software, which is part of the OpenAlea platform (Pradal et al. 2008), was used for extracting specific data from the MTG database. The database contained 17 and 20 branches, 3525 and 3464 GUs, and 1424 and 1491 metamers on medium and long GUs, respectively. This extraction procedure allowed the computation of (i) the number and types of GUs per year and per branch, (ii) the sequences of GU successions along second- and third-order axes, (iii) the number of metamers per year and per GU along the second-order axes, (iv) the frequency of axillary GU types along the second-order axes, (v) the proportion of third-order axes that stopped growing and died along each long and medium parent GU of the second-order axes, and (vi) the number of bourse shoots (one or two) following a floral GU.

Analysis of growth unit succession with VOMC - The sequences of GU types along axes were analyzed using variable-order Markov chains (VOMCs), introduced in the context of architectural modeling of apple trees by Costes and Guédon (2012). In VOMCs, GU type T_ℓ

at current position ℓ within the sequence is predicted by a stochastic process based on a finite and *a priori* bounded number k of previous GU types $T_{\ell-1},..,T_{\ell-k}$, where ℓ-1,... ℓ-k are the previous positions in the sequence. In this case, k may vary according to the values observed when following the sequence backwards. Transitions among GU types can be represented by a graph whose vertices are the subsequences $T_{\ell-1},..,T_{\ell-k}$ (also called *memories*) used to predict T_{ℓ}. The graph was adapted from the one used by Costes and Guédon (2012) on the 'Braeburn' cultivar. In this transition graph, the five previously defined GU types (L, M, F, S and D) were considered with D an absorbing state of the Markov chain. The transition frequencies were estimated with first-order (L, M, S and F0 for the initial flowering) or second-order memories (LF, MF, and SF for long-floral, medium-floral and short-floral). Therefore, the maximal value of k was 2 and the "LF" memory means that a floral GU was the predecessor and was preceded by a long GU ($T_{\ell-2}$=L and $T_{\ell-1}$=F). Based on this transition graph, the transition probabilities were estimated using the following multinomial logit model for the type $T_{t,m,w,\ell}$ of a GU of tree t at location ℓ in the tree, the memory at that location being m, for a water treatment w and considering S as the reference:

$$\log \frac{P(T_{t,m,w,\ell} = c)}{P(T_{t,m,w,\ell} = S)} = \lambda_c + \mu_{m,c} + \varphi_{w,c} + \theta_{m,w,c}, \quad (1)$$

where c is either L, M, F, D, λ_c is an intercept, $\mu_{m,c}$ is the effect on transition to type c of memory m (with references $\mu_{F_0,k} = 0$), $\varphi_{w,c}$ is the effect of water treatment w (with references $\varphi_{WS,c} = 0$), and $\theta_{m,w,c}$ is the effect of the interaction between memory m and water treatment w. Modeling the log ratio of the probabilities allows the probability of a categorical variable (comprised between 0 and 1) to be mapped into $\mathbb{R}$. The constraint that

the probabilities sum to one is ensured by modeling $P(T_{t,m,w,\ell} = S)$ implicitly as 1 minus the sum of all other probabilities. The model parameters were estimated by maximum likelihood using the VGAM package of R software (Yee 2010).

Five models were built: Model 1 took only the effect of memory into account, Model 2 took only the water treatment effect into account, Model 3 included both effects of memory and treatment, and Model 4 included both effects and their interaction. The significance of the different effects (water condition, predecessor and interaction) was assessed using a chi-squared likelihood ratio test, as suggested by Yee (2010). It consists in comparing the log likelihoods of the different models, with -2 loglikelihood(model0) + 2 loglikelihood(model1) following a chi-squared distribution under the null hypothesis with parameter df(model1)-df(model0), where loglikelihood(model) represents the log likelihood of a model and df(model) its number of free parameters. In this case, model0 and model1 refer to the null hypothesis and the alternative hypothesis, respectively. To assess whether the chosen significant level of the tests is sufficiently small, considering that the four models could all be incorrect and that the number of data is large, the models were also compared based on their Bayesian information criterion (BIC) (Kass and Raftery 1995), where the best model minimizes the BIC. BIC values were computed as follows:

$$BIC(\text{model}) = -2\text{loglikelihood}(\text{model}) + \text{df}(\text{model})\log(n) \quad (2)$$

where n is the number of data points.

The fifth model was obtained by adding a random "tree" effect to the best of the four previous models selected based on BIC values. This model was a multinomial logit model, whose inference can be achieved using the MCMCglmm package of R software (Hadfield

2010). A Bayesian analysis of the model was performed, using standard Gaussian assumptions for the random and fixed effects and under the assumption of independent components of fixed effects in the prior.

Data analysis at the branch scale. Mixed effect models considering branch as replicates, tree as random effect, treatment and year as fixed effects were fitted against the data collected at the branch scale. This procedure was carried out using the lme4 package of R software. Generalized linear models were used for proportions (binomial family) and for count variables displaying a small set of values (Poisson family). Linear models were used for count variables following a Gaussian distribution and which can be considered as continuous variables because they display a large set of values. The significance of fixed effects was then assessed using Chi-Square tests ('Anova' function of the car package of R software). The significance of the random tree effect was assessed by computing a confidence interval for its variance at level 95%. This was achieved by the 'confint' function of R software. The random effect was considered as significant if the lower bound of the confidence interval was higher than 1×10^{-2}.

Analysis of biennial bearing and variables at the whole tree scale - The impact of water treatment on whole tree variables (production variables and trunk diameter) was analyzed considering trees as replicates and using a two-way ANOVA with treatment and year effects and their interaction. The analysis was followed by a Student's t-test comparison, considering each year separately. Two indicators of the regular vs. irregular tree bearing

pattern were computed for each tree and for harvested fruit number and harvested fruit weight. As proposed by Durand et al. (2013), a first indicator (BBIR, for BBI on residuals) was computed. This indicator represents the Biennial Bearing Index computed on the residuals around the general trend over time and is adapted to take into account the increase in production during the first year of tree production into account. It was computed from the residuals ε_i of a linear regression of the considered variables X_i over time *i* (in years), as follows:

$$\text{BBIRN} = \frac{\sum_{i=2}^{y} \left| \varepsilon_i - \varepsilon_{i-1} \right| / \, y - 1}{\sum_{i=1}^{y} X_i \, / \, y}, \tag{3}$$

where *y* is the total number of years, ε_i is the Y-axis residual of the linear regression between the considered variable X_i and the year. A low value of BBIR is related to a regular bearing pattern, whereas a high value is related to an irregular bearing pattern. The second indicator was the Pearson correlation coefficient between two successive residuals ε_i and ε_{i+1}. This indicator (designated as cor_{res}) makes it possible to know if the irregular behavior observed with the BBIR is related to a biennial bearing pattern (cor_{res} values close to -1) or to an irregular behavior without any dependency between years (cor_{res} closer to 0). The treatment effect on cor_{res}, BBIR and the cumulative variables during the whole experiment (total yield and harvested fruit number) was assessed using Student's t-tests.

Results

Characterization of water stress intensity.

In this experiment, water stress was applied on half of the trees from 2007 to 2013. In 2008-2013, the soil water potential (mean and minimal values) of WS trees was lower than that of WW plants during summer and the beginning of autumn. WS trees reached minimal values between -0.20 and -0.25 MPa during this period, whereas observed values were between -0.02 and -0.17 MPa for WW trees (Figure 1, Supplementary Data, Figure S3). During the other months, the soil water potential of WS and WW plants were almost identical, with values ranging from 0 to -0.02 MPa. Lower differences were observed in 2007 (year of planting) for mean and minimal values of soil water potential, probably because trees were small and displayed a low transpiration demand. WS trees displayed a significantly lower trunk cross sectional area (TCSA) than WW trees. The mean values of TCSA were equal to 26.2 and 21.6 cm^2 for WW and WS plants in 2013, respectively (see Supplementary Data, Figure S4).

Primary growth: Mean number of metamers per GU and mean number of GUs per branch

Regardless of the type of GUs and the year, the number of metamers of long (20.0 ± 6.8 and 20.1 ± 4.7, for WW and WS, respectively) and medium (14.9 ± 4.7 and 13.5 ± 4.4) GUs did not show any difference between treatments (see Supplementary Data, Table S1) Conversely,

the total number of GUs developed per branch was significantly decreased by water stress (-16.4%, $P = 0.038$; Table 1). This decrease was observed regardless of the type of GU but was only significant ($P < 0.01$) for long and medium GUs, whereas the tree effect was only significant for the number of medium GUs. When considered as relative frequencies, the distribution of GUs was also affected by water stress, where WS trees had significantly ($P < 0.05$) relatively fewer long and medium GUs than WW trees. WW had a lower proportion of floral GUs but this difference was non-significant ($P = 0.064$). Conversely, the relative frequency of short GUs was similar for both treatments. Tree effect was significant for all GU proportions, except long GUs.

Dynamics of GU types over the years

The number of GUs produced per year showed a significant year effect ($P < 0.001$) for all types of GUs (Figure 2). The treatment effect was only significant on the dynamics of long and medium GUs (Figure 2A, $P < 0.001$), and the interaction between treatment and year effects was only significant for floral GUs (Figure 2C, $P = 0.003$). Tree effect was significant only in specific years.

For both treatments, the mean number of long and medium GUs per branch continuously increased from 2008 (second year of tree growth) until 2010. Then, it dramatically decreased in 2011 (fifth year of tree growth). From 2011 to 2013, the number of long and medium GUs alternated with years, with low values in 2011 and 2013 and medium values in 2012. In the last year (2014), the number of long and medium GUs was close to 0 (Figure 2A). In contrast, the mean number of floral GUs increased until 2011 (Figure 2C)

and then exhibited a biennial fluctuation. If we compare fluctuations of long and medium GUs with floral GUs during the 2011-2013 period, we can observe that years with high values of floral GUs (2011, 2013) correspond to years of low values of long-medium GUs. Conversely, 2012, which was a year of high production of long and medium GUs, was a year of low floral GU production.

The production of short GUs per branch exhibited an increase over the years, especially in WW trees, without any decrease as observed for long and medium GUs (Figure 2B). A strong increase in the number of short GUs was observed in 2011 (fifth year of tree growth). From 2008 until 2010, the mean number of short GUs per branch was equal to 3.65 and 3.40 for WW and WS, respectively. From 2011 until 2014, this number was equal to 21.34 and 17.76 for WW and WS, respectively.

If each year was considered separately in the analysis, significant differences ($P < 0.05$) between treatments were observed from the second year (2008) until the end of the experiment for the long and medium GUs. For floral and short GUs, differences between treatments remained non-significant for most of the years, except in 2013 when a significantly ($P = 0.008$) greater number of floral GUs was observed for WW trees.

Transitions between GU types along the axes

The comparison of the logit multinomial models (see Supplementary Data, Table S2) showed that the "memory" effect on the type of GU produced along the axes was more significant (BIC = 9,465 for Model 1 including the memory effect alone) than the "water conditions" effect (BIC = 14,894 for Model 2 including the water stress treatment alone).

The higher significance of the "memory" effect compared to "water condition" was also observed by testing their significance in Model 3, which includes both kinds of effect. The *P*-value associated with testing the absence of "memory" effect (chi-squared likelihood ratio test) was lower than 10^{-324}, compared to 2.9×10^{-5} for "water condition". The latter *P*-value suggested that the "water condition" effect was highly significant. However, the BIC value was higher for Model 3 (9,474) than for Model 1; thus the impact of water conditions on transition frequencies was actually moderate.

According to the chi-squared likelihood ratio test applied to assess the significance of treatment and memory interaction effects in Model 4, this interaction was non-significant (*P* = 0.24). This test was in favor of Model 3 as opposed to Model 4, which was consistent with its BIC value (9,655). Thus, parameters associated with the "memory" effect (as estimated in Supplementary Data, Table S3) were not changed by water stress or, in other words, the effect of water stress was independent of the memory. As a consequence, Model 3 was chosen as a reference. A random individual "tree" effect was added to Model 3, leading to Model 5. To assess the significance of the tree effect, a Bayesian estimation of Model 5 was performed. A 95% credibility interval was computed for the posterior variance distribution: [lower bound = 0.005, upper bound = 0.162], showing that variability in the transition probabilities due to the tree effect was not significant. As a consequence, subsequent inference was based on Model 3.

The odd ratio estimates of the model with additive water treatment and memory effects (Model 3, taking type S as a reference; see Supplementary Data, Table S3) showed an increase, under well-watered conditions, in the relative frequencies of transition towards

long (L, odds ratio × 1.39; $P > 0.05$) and medium GUs (M, odds ratio × 1.34; $P = 0.013$) and a decrease in the relative frequencies of transitions towards dead (D, odds ratio × 0.76; $P < 0.001$) and floral (F, odds ratio × 0.81; $P = 0.014$) GUs, regardless of the type of predecessor. Using M as a reference, the model showed a decrease in the relative frequencies of transition towards short GUs (S, odds ratio × 0.75; $P = 0.012$), under well-watered conditions. Finally, the increase in transition frequencies towards long and medium GUs at the expense of transition towards floral, short and dead GUs observed under well-watered conditions was consistent with the direct estimation of the transition frequencies between GUs shown in Figure 3 and with the estimated frequencies of each GU type produced per branch (Table 1). Moreover, a decrease in the transition towards dead GUs (D, odds ratio × 0.72; $P = 0.016$) after an initial floral GU on the axis (F0 state) was observed for WW trees. We also analyzed the number of bourse shoots that arose from floral GUs. Water stress significantly decreased the frequency of floral GUs with two bourse shoots (15% for WW and 10% for WS).

Branching characteristics: Axillary bud fate and axis death along parent GUs

The two treatments did not display any significant difference for the total number of axillary GUs along parent GUs, regardless of their type (long or medium, Table 2). Under WS conditions, long parent GUs had relatively more axillary floral GUs and less latent buds, and the treatment effect was close to being significant at $P < 0.05$ ($P = 0.052$ and 0.058 for latent buds and floral axillary GUs, respectively). Conversely, the proportions of short axillary GUs were quite similar between treatments. For the medium parent GUs, the frequency of the different axillary GUs was similar for the two treatments (Table 2). A significant tree

effect was observed for the short and floral axillary GUs on long and medium parent GUs and for the number of axillary GUs and the proportion of latent buds on medium parent GUs.

The cumulated death frequency of third-order axes was then calculated according to the age of the parent GU along the second-order axes (Figure 4). A significant effect ($P < 0.001$) of the age of the parent GU on this frequency was observed, and this frequency followed a linear increase with the parent GU's age. No significant impact of water stress on axis death frequency was observed although the death probability was slightly higher under WS, regardless of the age of the parent GU. No significant tree effect was observed for the changes in axis death according to parent GU age.

Tree production and bearing behavior

The cumulative total number of fruits per tree during this experiment was higher under WS but this effect was non-significant ($P = 0.26$; Table 3) and the cumulative yield was roughly similar between WW and WS tress.

The two-way ANOVA (year and treatment effect) showed a significant year effect on production variables ($P < 0.01$; Figure 5). In the first year of tree production in 2010 (fourth year of tree growth), the number of harvested fruits and the fruit weight were close to zero for both treatments. 2011 and 2013 appeared to be years of high production (ON years), whereas the production in 2012 and 2014 was low (OFF years).The water treatment effect was only significant for the mean individual fruit weight ($P = 0.014$), which was equal to 153 and 122 g on average during the whole period for WW and WS, respectively (Figure

5C). The interaction between year and treatment effects was significant for yield ($P = 0.007$) alone. Indeed, in ON years, yield was greater for WW trees, whereas in OFF years, yield was greater for WS plants (Figure 5A). A significantly ($P < 0.05$) higher fruit number per tree was also observed in OFF years for WS trees, whereas it was roughly similar for both treatments in ON years (Figure 5B). Crop load was significantly affected by years ($P < 0.001$) and treatments ($P = 0.02$) and a significantly greater crop load ($P < 0.05$) was observed in OFF years (2012 and 2014) for WS trees (Figure 5D). A covariance analysis with crop load and treatment effects was carried out on individual fruit weight and considering years 2012, 2013 and 2014 together. This analysis revealed a significant effect of crop load ($P = 0.001$) and an absence of significant impact of treatment ($P = 0.81$), showing that the decrease in individual fruit weight under WS (Figure 5C) was statistically explained by an increase in crop load.

The value of the slopes of the linear regressions between harvested fruit number and year (+4.6 fruit year^{-1}) and between yield and year (+0.51 kg year^{-1}) did not show any large increasing trend in yield and harvested fruit number with tree age. Consequently the general trend over time used to compute BBIR and cor_{res} for harvested fruit number and yield roughly corresponds to the mean observed values of both variables. BBIR was significantly ($P<0.05$) lower for the number of fruits per tree and yield under WS (Table 3). In the same way, the cor_{res} was significantly ($P<0.05$) lower under WS for both variables. These results are consistent with the attenuated irregular bearing pattern observed for WS trees compared to WW trees (Figure 5).

Discussion

Ontogeny and successive developmental phases

'Granny Smith' branches exhibited two distinct development phases, one from the second (2008) to the fourth (2010) year of tree growth and the second one starting in the fifth year (2011; Figure 2). The existence of distinct developmental phases has been previously reported in other apple cultivars, e.g., 'Fuji' and 'Braeburn' (Costes and Guédon 2012). In this previous study, the two phases were referred to as 'adolescent' and 'adult'. The first phase ("adolescent") was characterized by the occurrence of long GUs and the beginning of flower production because the use of dwarf rootstock makes it possible to obtain flowers not long after planting (Seleznyova et al. 2008). The second phase ("adult") included patterns of alternation between vegetative and reproductive GUs.

In our study, the first phase was also characterized by the occurrence of long GUs along the axes, but because the long first GUs of the trunks (first year of growth) were not included in the analysis, it only partially corresponds to the adolescent phase. Thereafter, the long GUs were progressively replaced by medium GUs (data not shown), whereas the number of floral GUs increased. The strong decrease in the long and medium GUs number concomitant to the maximum floral GUs number in the fifth year indicates the starting point of alternation at the whole tree level. The second phase (adult phase) was then characterized by the alternation of floral GUs between consecutive years, concomitant with fluctuations in the number of long and medium GUs. During the last year (eighth year of tree growth), in

spite of the low production of floral GUs, the number of long and medium GUs remained low, which is consistent with the morphogenetic gradients characterized by a decrease in primary GU length with plant age (Costes et al. 2003).

This study also reveals that the two development phases ('adolescent' and 'adult', as designated by Costes and Guédon (2012)) are observed independently of the soil water status. This confirms the stability of the ontogenetic characteristics of plants, even if they are subjected to environmental stresses (Barthélémy and Caraglio 2007). Despite 'Granny Smith' being considered as a regular cultivar in agronomic conditions (Lespinasse 1992), this study shows that it could display years of high floral production followed by years of low production that can lead to alternate bearing at the tree scale (Figure 5). This divergence in the bearing behavior may be due to the absence of pruning practices in our experiment (Jonkers 1979). Nevertheless, the intensity of biennial bearing was lower than for other apple tree genotypes that displayed yield values close to 0 in OFF years (Lauri et al. 1995, Durand et al. 2013). Indeed, in our study, yield in OFF years was almost equal to one-third and two-third of the yield in ON years for WW and WS trees, respectively.

Water stress effect on vegetative GU development

Water stress significantly decreased the total number of GUs (Table 1), the proportion of long and medium GUs and increased the transition probability towards small and dead GUs (Figure 3, Supplementary Data, Table S3). This suggests an acceleration of the ontogenetic gradient as observed under stressful conditions on walnut (Taugourdeau et al. 2011) or almond (Negron et al. 2014). The decrease in the length of vegetative GUs and the lower

number of GUs led to a reduction in tree vigor under water stress, as revealed by the decrease in the trunk cross-sectional area (Supplementary Data, Figure S4). Nevertheless, since metamer numbers on long and medium GUs were almost the same between treatments (Supplementary Data, Table S1) and because no data were collected on internode length, changes in GU length could be only analyzed through changes in GU types.

The decrease in the proportion of long GUs under WS (Table 1) suggests that the capability to develop neoformed metamers was hampered in early summer, at least in the first years of tree development when neoformation occurred. Such a direct impact of water stress could result from an increase in the phyllochron or from an early arrest of leaf production by apical meristems, as observed in maize and grapevine (Reymond et al. 2003, Lebon et al. 2006, respectively).

Water stress could also have modified GU growth potential through a one-year delayed effect. This delayed effect could be associated with a reduction in shoot elongation after budburst the year following stress conditions, as observed in previous studies considering trees subjected to summer water stress (almond; Esparza et al. 2001) or early defoliation in autumn (apple; Tustin et al. 1997) leading to reduce the proportion of long and medium vegetative GUs under water stress.

The mortality of terminal buds was increased under water stress (Figure 3, Supplementary Data, Table S3). Bud death was observed after both vegetative and floral GUs and only partially corresponded to the extinction phenomenon defined as the absence or non-development of a bourse shoot (Lauri et al. 1995). It has been suggested that bud mortality results from hydraulic mechanisms such as embolism, breaking the connection to

the buds, or from an inability of the bourse shoot buds to attract assimilate from sources (Lauri et al. (1995, 1997) on apple and Barigah et al. (2013) on poplar and beech).

In our study, WS had no clear impact on the type and number of vegetative axillary GUs (Table 2). This absence of effects contrasts with the results obtained in grapevine by Lebon et al. (2006) and Pallas and Christophe (2015) who showed that sylleptic branching is the growth process that accounts for the major part of the decrease in total leaf area under water stress. In that species, which is a liana, water stress reduced the duration of shoot neoformation as well as the leaf production rate. In our case, no syllepsy and little neoformation occurred since a majority of the axillary buds producing vegetative GUs produced short GUs in both treatments. This is probably due to the characteristics of the cultivar and to the fact that the first GUs of the trunk that display many long sylleptic GUs were not considered in this study, thus limiting our ability to observe the effects of WS on syllepsis and axillary bud neoformation.

Water stress affects flowering occurrence, biennial bearing and yield components

An increase in the proportion of reproductive GUs was observed under water stress as shown by the increase in the proportion of floral GUs along the axes (Table 1) and by the transition probabilities that revealed a higher probability of transition towards floral GUs under water stress (Figure 3).

In apple, a negative correlation between crop load in year n and flowering in year n +1 is usually observed (Singh 1948, Jonkers 1979). Both hormonal and trophic hypotheses have been proposed to explain this dependency (Wilkie et al. 2008, Bangerth 2009). According to

the hormonal hypothesis, floral induction is inhibited by gibberellin signaling from seeds, and (ii) according to the trophic hypothesis, floral induction is limited by carbon availability. If we consider ON years (2011 and 2013) in which the number of developing fruits and the crop load (only measured in 2013) were almost the same for both treatments (Figure 5), the number of fruits and the floral induction rate should have been identical in the following year. However, a higher fruit number was observed under WS in the next years, which were OFF (2012, 2014), reducing the biennial bearing pattern (Table 3) and suggesting that other mechanisms could be involved.

One hypothesis to explain this is related to the decrease in vegetative growth under WS, as observed with the change in GU type proportions and in the total number of GUs (Table 1). This reduced vegetative growth could reflect non-trophic mechanisms, as observed under moderate stress before any effect of water stress on the photosynthesis activity (Tardieu et al. 1999, Muller et al. 2011). In turn, this reduced vegetative growth could favor plant growth processes such as floral induction and fruit set. This assumption is in line with other results showing that excessive vegetative vigor is antagonistic to flower bud initiation in apple (Forshey and Elfving 1989) and other temperate fruit trees (Faust 1989). It is also in accordance with the observed increase in floral induction when apple trees are grafted on dwarfing rootstock with low vegetative development (Seleznyova et al. 2008). In that case, they also displayed a more regular bearing pattern (Costes and Garcia-Villanueva 2007).

Similarly, lower vegetative growth during the fruit set period might also have increased the fruit set under WS. This could explain the relative dissimilarity between the number of floral GUs recorded at the branch scale and the number of harvested fruits at the tree scale.

Indeed, despite similar numbers of floral GUs in WW and WS trees at the branch scale in 2012 and 2014 (OFF years) and lower ones in 2013 (ON year, Figure 2), the number of harvested fruits was higher on WS trees in OFF years and was almost identical between treatments in ON years, thus suggesting that the fruit set was higher under WS. Such an impact of excessive vegetative development on fruit/seed set has been reported on other woody plants such as eucalyptus (Suitor et al. 2010).

Finally, individual fruit weight was reduced under water stress conditions (Figure 5). Our study suggests that the decrease in fruit weight under WS was associated with an increase in crop load. Such a direct impact of crop load on fruit growth was observed in many fruit species such as peach (Berman and DeJong 1996) and apple (Girona et al. 2010). Nevertheless, the absence of photosynthesis measurement in our study did not allow us to clearly determine if the observed impact is only due to an increase in crop load or could be also associated with a decrease in photosynthesis activity under WS.

Conclusion

The multi-scale analysis performed in this study revealed that water stress affected the total number of GUs, the transition frequencies between the different GU types, and the equilibrium between vegetative and reproductive growth. Water stress mainly increased the rate of reduction in the size of GUs during tree ontogeny and increased the probability of bud death. Water stress also affected the recurrent transition from vegetative to reproductive

GUs by maintaining a more constant proportion of floral GUs every year compared to control conditions. This led to decreased biennial bearing at the tree scale. The study also provides information that could be relevant for modeling. For instance, variable-order Markov chains with parameters that depend on environmental conditions could be integrated into the functional-structural plant model MappleT (Costes et al. 2008). Such improvements could be an interesting way to further analyze the impact of modifications in GU successions and branching at the shoot scale on biennial bearing under contrasted environmental conditions and could open new perspectives for *in silico* investigations of agronomical scenarios.

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Conflict of interest

None declared

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Figures

Figure 1. Mean and minimal soil water potential between 1st April and 15th September for each year under well-watered (WW) and water stress (WS) treatments during the experiment. (A) Manual measurements performed in 2011-2013 at a depth of 30 cm. (B) Automatic measurements performed in 2007–2013, except in 2011 at a depth of 30 cm. Bars represent standard deviations between trees.

Figure 2. Mean number of growth units (GU) per branch under well-watered (WW) and water stress (WS) treatments during the experiment according to GU types. (A) Long and medium GUs. (B) Short GUs. (C) Floral GUs. A generalized linear mixed effect model (Poisson family) including the year and treatment effects and their interaction as fixed effects and tree as a random effect was fitted against the data for each type of GU. The significance of the fixed effects estimated using a Chi-square test is represented in the top left corner. For each year, a generalized linear model mixed effect model (Poisson family) with treatment as a fixed effect and tree as a random effect was used, and the significance of the treatment effect is represented above the dots. *, ** and *** indicate significant differences at $P < 0.05$, $P < 0.01$ and $P < 0.001$ levels and ns indicates no significant differences at the $P < 0.05$ level. For each year, no significant difference between treatments was detected if no sign is represented. t above dots represents cases for which tree effect was significant (considering a confidence interval of 5%). Bars represent standard deviations between trees.

Figure 3. Transition frequencies between GUs under well-watered (WW, black numbers) and water stress (WS, gray numbers) treatments. The transition frequencies were estimated from first- (L, M, S and D for long, medium, short and dead GU and F0 for the initial flowering) and second-order memories (LF, MF and SF for long-floral, medium-floral and short-floral). The transition frequencies lower than 0.05 were not represented. Circles and arrows represented by dashed lines correspond to transition and memories that can be observed only once in the GU sequences after an initial floral GU. Continuous lines and circles correspond to memories and transition that can occur many times in the GU sequence. Double circles indicate an absorbing state (D, dead) of the Markov chain.

Figure 4. Relationships between parent growth unit (second-order axis) age and frequency of axillary shoot death (death of third-order axis) under water stress (WS) and well-watered (WW) treatments. Lines represent the regression line for WW (solid line, $y = 0.193x$ 0.286, $R^2 = 0.96$), and WS (dashed line, $y = 0.171x - 0.231$, $R^2 = 0.97$). A generalized linear mixed effect model (binomial family) including the year and treatment effects and their interaction as fixed effects and tree as a random effect was fitted against the dataset. The significance of the fixed effects estimated using a Chi-square test is represented in the top left corner. For each year, a generalized linear mixed effect model (binomial family) with treatment as a fixed effect and tree as a random effect was used, and the significance of the treatment effect is represented above the dots. *** indicates significant differences at the $P < 0.001$ level, and ns indicates no significant differences between treatments at $P < 0.05$ level. t above dots

represents cases for which tree effect was significant (considering a confidence interval of 5%). Bars represent standard deviations between trees.

Figure 5. Yield (A), number of fruits per tree (B), mean individual fruit weight (C), and crop load (number of fruits per unit trunk cross-sectional area, D) under well-watered (WW) and water stress (WS) treatments. Crop load was not shown in 2010-2011 because trunk diameter was not measured during this period. A two-way ANOVA considering year and treatment effects and their interaction was performed, and the significance of the P-value (F-test) associated with each effect is represented in the top left corner of each graph. The analysis was followed by a Student's t-test, considering each year separately. The significance of the *P*-value associated with these Student's t-tests is represented above the dots if significant differences have been detected. *, ** and *** indicate significant differences at $P < 0.05$, $P < 0.01$ and $P < 0.001$ levels, and ns indicates no significant differences at the $P < 0.05$ level. Bars represent standard deviations between trees.

Supplementary Data

Figure S1. Mean, minimum and maximum values for daily temperature (A), maximal daily vapor pressure deficit (VPD) (B), mean daily solar radiation (C) and total rainfall during the growing period (1 April – 15 September D) for each year of the experiment.

Figure S2. Schematic representation of a 7-year-old branch at the GU scale. GUs of each axis are labelled according to their growing year. Vegetative GUs were classified based on their length: Long GUs (designated as L, length > 20 cm), Medium GUs (designated as M, 5 cm < length < 20 cm); Short GUs (designated as S, length < 5 cm). Floral GUs were designated as F and dead GUs were designated as D. The GU sequence along the second-order axis of this branch example is LFLFLFSLFSM.

Figure S3. Seasonal variations in daily soil water potential during the growing year for 'Granny Smith' under well-watered (WW) and water stress (WS) treatments during the experiment. Automatic measurements were not performed in 2011 due to technical problems. Gray and black lines represent values recorded at a depth of 30 cm for WS and WW trees , respectively.

Figure S4. Evolution of trunk cross-sectional area during the experiment under well-watered (WW) and water stress (WS) treatments. A two-way ANOVA considering year and treatment effects and their interaction was performed and the significance of each effect is

represented in the bottom left corner of the graph. The analysis was followed by a Student's t-test comparison, considering each year separately. The significance of the *P*-values associated with these Student's t-tests is represented above the dots if significant differences have been detected. *, ** and *** indicate significant differences at the $P < 0.05$, $P < 0.01$ and $P < 0.001$ levels, and ns indicates no significant differences at the $P < 0.05$ level. Bars represent the standard deviation between trees.

Table 1. Mean number of growth units (GU) per branch and frequencies of the different types of GU under well water (WW) and water stress (WS) treatments. The associated *P*-values of the treatment effect of the mixed effect models including a fixed treatment effect and a tree random effect are represented. Statistical analyses were performed using linear models for the total number of GUs and for the number of short and floral GUs. Generalized linear models of the Poisson family were used for the number of long and medium GUs and generalized linear models of the binomial family were used for GU frequencies. *P*-values in bold are significant at $P < 0.05$.

	GU number					GU frequencies			
Treatment	Long	Medium	Short	Floral	Total	Long	Medium	Short	Floral
WW	6.3	13.9	97.4	89.8	207.4	0.031	0.067	0.468	0.434
WS	4.0	7.8	81.7	79.9	173.4	0.024	0.042	0.474	0.460
P-values (treatment effect)	**0.002**	**0.008** [t]	0.072	0.430	**0.038**	**0.049**	**0.021** [t]	0.96 [t]	0.064 [t]

t indicates cases for which tree random effect was significant (considering a confidence interval of 5%).

Table 2. Mean number of axillary GUs per growth unit and axillary bud fate under well water (WW) and water stress (WS) treatments for long and medium parent GUs. The associated *P*-values of the treatment effect of the mixed effect models including a fixed treatment effect and a tree random effect are represented. Statistical analyses were performed using generalized linear models of the Poisson family for the total number of axillary buds and generalized linear models of the binomial family for frequencies.

Parent GU type	Treatment	Mean number of axillary GUs	Axillary bud fate (frequencies)			
			Latent buds	Long & Medium GUs	Short GUs	Floral GUs
Long	WW	9.4	0.587	0.009	0.053	0.351
	WS	8.2	0.536	0.003	0.05	0.411
	P-values (treatment effect)	0.72	0.052	0.33	0.97[t]	0.058[t]
Medium	WW	4.6	0.738	0.007	0.092	0.163
	WS	4.1	0.730	0.003	0.112	0.155
	P-values (treatment effect)	0.65[t]	0.052[t]	0.38	0.79[t]	0.71[t]

t indicates cases for which tree effect was significant (considering a confidence interval of 5%).

Table 3. Cumulative values during the whole experiment, BBIRN and cor_{res} for yield and number of fruits per tree under well water (WW) and water stress (WS) treatments. Student's *t*-test comparisons were performed and the *P*-values are represented. *P*-values in bold are significant at $P < 0.05$.

	Cumulative value			BBIRN			cor_{res}		
	WW	WS	Treatment effect (*P*-values)	WW	WS	Treatment effect (*P*-values)	WW	WS	Treatment effect (*P*-values)
Yield (kg tree^{-1})	70.5	68.3	0.820	1.46	0.87	**0.022**	-0.82	-0.54	**0.028**
Number of fruits per tree	534.0	611.0	0.261	1.69	1.08	**0.034**	-0.91	-0.62	**0.017**

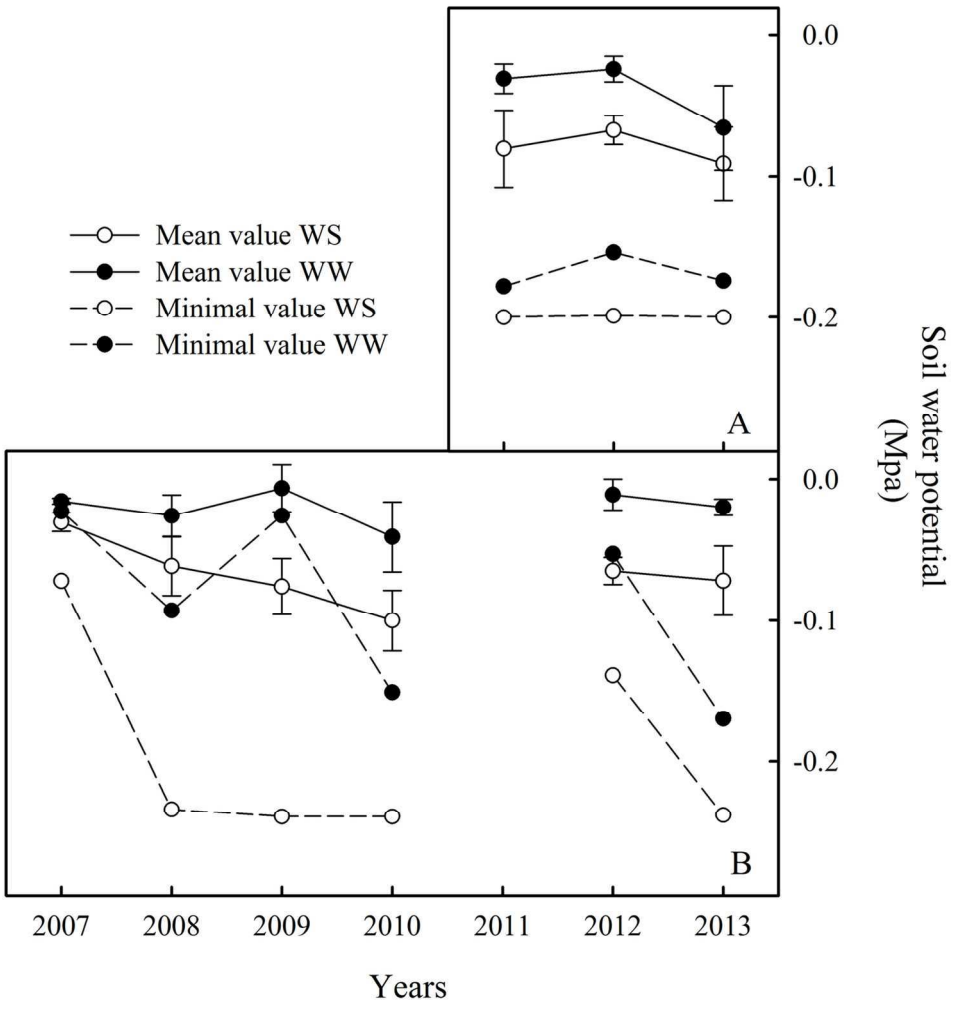

Figure 1. Mean and minimal soil water potential between 1st April and 15th September for each year under well-watered (WW) and water stress (WS) treatments during the experiment. (A) Manual measurements performed in 2011-2013 at a depth of 30 cm. (B) Automatic measurements performed in 2007–2013, except in 2011 at a depth of 30 cm. Bars represent standard deviations between trees.

131x143mm (300 x 300 DPI)

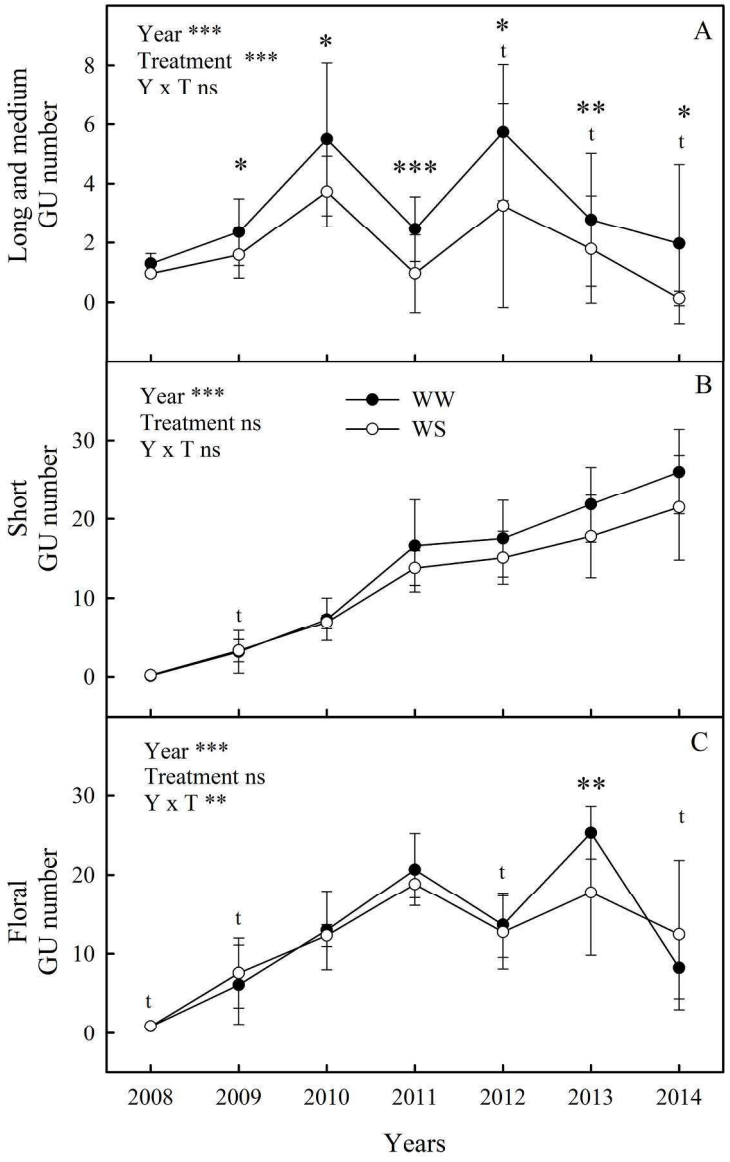

Figure 2. Mean number of growth units (GU) per branch under well-watered (WW) and water stress (WS) treatments during the experiment according to GU types. (A) Long and medium GUs. (B) Short GUs. (C) Floral GUs. A generalized linear mixed effect model (Poisson family) including the year and treatment effects and their interaction as fixed effects and tree as a random effect was fitted against the data for each type of GU. The significance of the fixed effects estimated using a Chi-square test is represented in the top left corner. For each year, a generalized linear model mixed effect model (Poisson family) with treatment as a fixed effect and tree as a random effect was used, and the significance of the treatment effect is represented above the dots. *, ** and *** indicate significant differences at $P < 0.05$, $P < 0.01$ and $P < 0.001$ levels and ns indicates no significant differences at the $P < 0.05$ level. For each year, no significant difference between treatments was detected if no sign is represented. t above dots represents cases for which tree effect was significant (considering a confidence interval of 5%). Bars represent standard deviations between trees.

184x284mm (300 x 300 DPI)

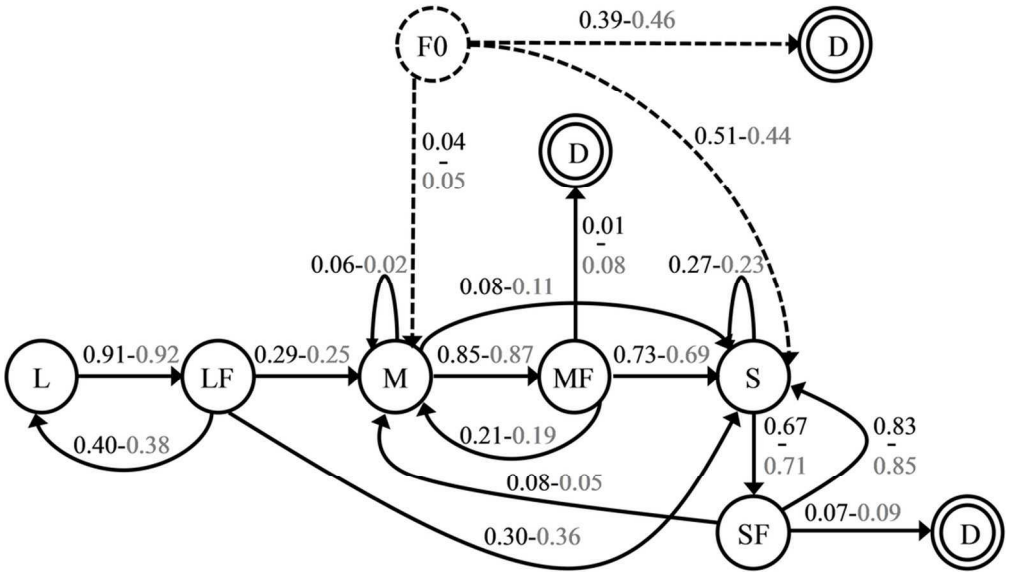

Figure 3. Transition frequencies between GUs under well-watered (WW, black numbers) and water stress (WS, gray numbers) treatments. The transition frequencies were estimated from first- (L, M, S and D for long, medium, short and dead GU and F0 for the initial flowering) and second-order memories (LF, MF and SF for long-floral, medium-floral and short-floral). The transition frequencies lower than 0.05 were not represented. Circles and arrows represented by dashed lines correspond to transition and memories that can be observed only once in the GU sequences after an initial floral GU. Continuous lines and circles correspond to memories and transition that can occur many times in the GU sequence. Double circles indicate an absorbing state (D, dead) of the Markov chain.

98x56mm (300 x 300 DPI)

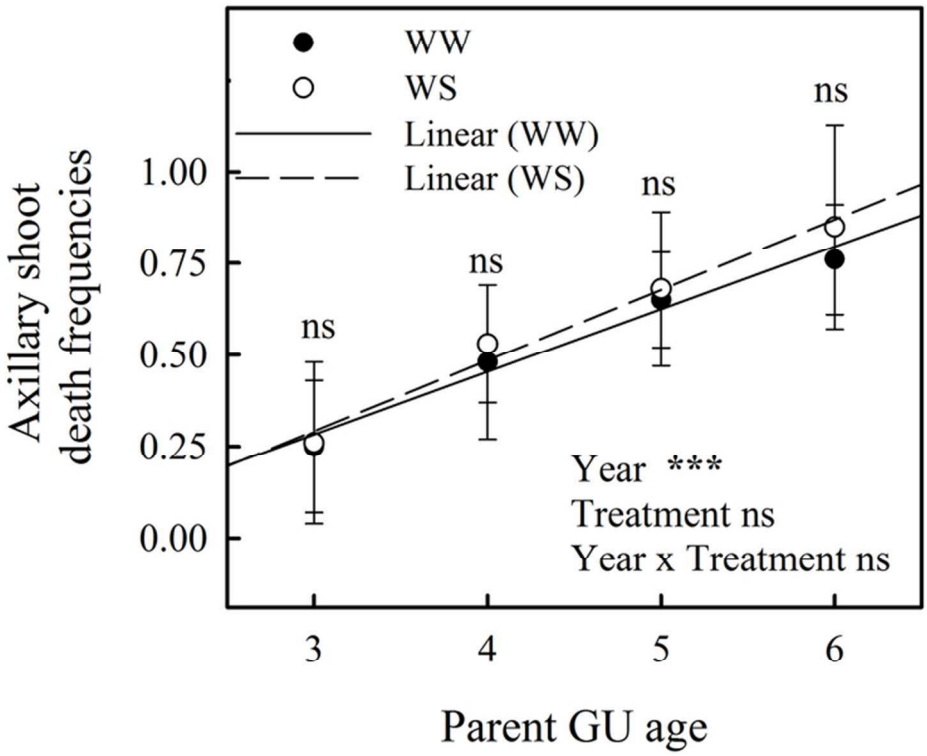

Figure 4. Relationships between parent growth unit (second-order axis) age and frequency of axillary shoot death (death of third-order axis) under water stress (WS) and well-watered (WW) treatments. Lines represent the regression line for WW (solid line, y = 0.193x 0.286, R^2 = 0.96), and WS (dashed line, y = 0.171x – 0.231, R^2 = 0.97). A generalized linear mixed effect model (binomial family) including the year and treatment effects and their interaction as fixed effects and tree as a random effect was fitted against the dataset. The significance of the fixed effects estimated using a Chi-square test is represented in the top left corner. For each year, a generalized linear mixed effect model (binomial family) with treatment as a fixed effect and tree as a random effect was used, and the significance of the treatment effect is represented above the dots. *** indicates significant differences at the P < 0.001 level, and ns indicates no significant differences between treatments at P < 0.05 level. t above dots represents cases for which tree effect was significant (considering a confidence interval of 5%). Bars represent standard deviations between trees.

67x55mm (300 x 300 DPI)

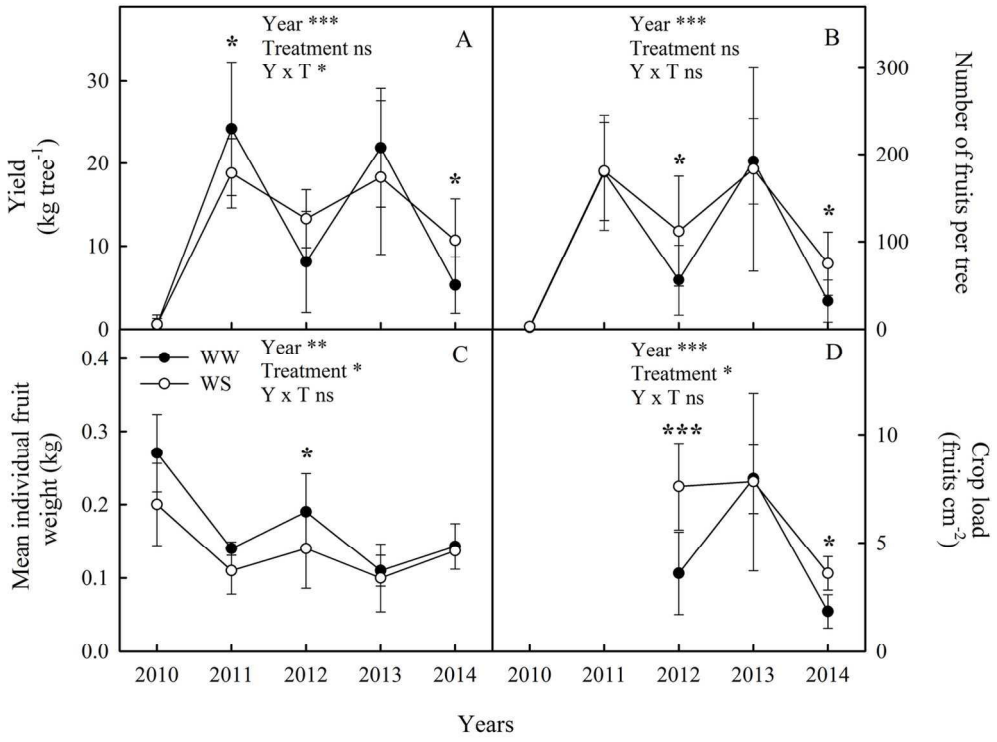

Figure 5. Yield (A), number of fruits per tree (B), mean individual fruit weight (C), and crop load (number of fruits per unit trunk cross-sectional area, D) under well-watered (WW) and water stress (WS) treatments. Crop load was not shown in 2010-2011 because trunk diameter was not measured during this period. A two-way ANOVA considering year and treatment effects and their interaction was performed, and the significance of the P-value (F-test) associated with each effect is represented in the top left corner of each graph. The analysis was followed by a Student's t-test, considering each year separately. The significance of the P-value associated with these Student's t-tests is represented above the dots if significant differences have been detected. *, ** and *** indicate significant differences at $P < 0.05$, $P < 0.01$ and $P < 0.001$ levels, and ns indicates no significant differences at the $P < 0.05$ level. Bars represent standard deviations between trees.

132x102mm (300 x 300 DPI)